

EXAMPLE 16**5'-O-DMT-3'-O-methoxyethyl-N²-isobutyryl-guanosine-2'-O-succinoyl Linked LCA CPG**

[0151] Following succinylation, 5'-O-DMT-3'-O-(2-methoxyethyl)-2'-O-succinyl-N²-benzoyl guanosine was coupled to controlled pore glass (CPG). 3.42 g (4.20 mmol) of the succinate were dried overnight in a vacuum oven along with 4-dimethylaminopyridine (DMAP), 2,2'-dithiobis (5-nitro-pyridine) (dTNP), triphenylphosphine (TPP), and pre-acid washed CPG (controlled pore glass). The following day, DMAP (4.20 mmol, 513 mg) and acetonitrile (37.5 mL) were added to the succinate. The mixture was "mixed" by a magnetic stirrer under argon. In a separate flask, dTNP (4.20 mmol, 1.43 g) was dissolved in acetonitrile (26 mL) and dichloromethane (11 mL) under argon. This reaction mixture was then added to the succinate. In another separate flask, TPP (4.20 mmol, 1.10 g) was dissolved in acetonitrile (37.5 mL) under argon. This mixture was then added to the succinate/DMAP/dTNP reaction mixture. Finally, 33.75 g pre-acid washed LCA CPG (loading = 115.2 $\mu\text{mol/g}$) were added to the main reaction mixture, vortexed shortly and placed on shaker for approximately 20 hours. Removed from shaker after 20 hours and the loading was checked. A small sample of CPG was washed with copious amounts of acetonitrile, dichloromethane, and then with ether. The initial loading was found to be 64 $\mu\text{mol/g}$. (3.4 mg of CPG were cleaved with trichloroacetic acid). The absorption of released trityl cation was read at 503 nm on a spectrophotometer to determine the loading. The CPG was then washed as described above and dried under P₂O₅ overnight in vacuum oven. The following day, the CPG was capped with 50

mL CAP A (tetrahydrofuran/acetic anhydride) and 50 mL CAP B (tetrahydrofuran/pyridine/1-methylimidazole) for approximately 1 hour on a shaker. The material was filtered and washed with dichloromethane and ether. The CPG was dried under P_2O_5 overnight in vacuum oven. After drying, 33.75 g. of CPG was isolated with a final loading of 72 $\mu\text{mol/g}$.

EXAMPLE 17

5'-O-DMT-3'-O-[hexyl-(6-phthalimido)]-uridine

[0152] 2',3'-O-Dibutyl stannylene-uridine was synthesized according to the procedure of Wagner *et. al.*, *J. Org. Chem.*, 1974, 39, 24. This compound was dried over P_2O_5 under vacuum for 12 hours. To a solution of this compound (29 g, 42.1 mmol) in 200 mL of anhydrous DMF were added (16.8 g, 55 mmol) of 6-bromohexyl phthalimide and 4.5 g of sodium iodide and the mixture was heated at 130 °C for 16 hours under argon. The reaction mixture was evaporated, co-evaporated once with toluene and the gummy tar residue was applied on a silica column (500 g). The column was washed with 2 L of EtOAc followed by eluting with 10% methanol (MeOH):90% EtOAc. The product, 2'- and 3'-isomers of O-hexyl-6-N-phthalimido uridine, eluted as an inseparable mixture ($R_f=0.64$ in 10% MeOH in EtOAc). By ^{13}C NMR, the isomeric ration was about 55% of the 2' isomer and about 45% of the 3' isomer. The combined yield was 9.2 g (46.2%). This mixture was dried under vacuum and re-evaporated twice with pyridine. It was dissolved in 150 mL anhydrous pyridine and treated with 7.5 g of DMT-Cl (22.13 mmol) and 500 mg of dimethylaminopyridine (DMAP). After 2 hours, thin layer chromatography (TLC; 6:4 EtOAc:Hexane) indicated complete disappearance of the

starting material and a good separation between 2' and 3' isomers ($R_f=0.29$ for the 2' isomer and 0.12 for the 3' isomer). The reaction mixture was quenched by the addition of 5 mL of CH_3OH and evaporated under reduced pressure. The residue was dissolved in 300 mL CH_2Cl_2 , washed successively with saturated NaHCO_3 followed by saturated NaCl solution. It was dried over Mg_2SO_4 and evaporated to give 15 g of a brown foam which was purified on a silica gel (500 g) to give 6.5 g of the 2'-isomer and 3.5 g of the 3' isomer.

EXAMPLE 18

5'-O-DMT-3'-O-[hexyl-(6-phthalimido)]-uridine-2'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite

[0153] 5'-DMT-3'-O-[hexyl-(6-phthalimido)]uridine (2 g, 2.6 mmol) was dissolved in 20 mL anhydrous CH_2Cl_2 . To this solution diisopropylaminotetrazolide (0.2 g, 1.16 mmol) and 2.0 mL 2-cyanoethyl-N,N,N',N'-tetraisopropyl phosphoramidite (6.3 mmol) were added with stirred overnight. TLC (1:1 EtOAc/hexane) showed complete disappearance of starting material. The reaction mixture was transferred with CH_2Cl_2 and washed with saturated NaHCO_3 (100 mL), followed by saturated NaCl solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to yield 3.8 g of a crude product, which was purified in a silica column (200 g) using 1:1 hexane/EtOAc to give 1.9 g (1.95 mmol, 74% yield) of the desired phosphoramidite.